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Analysis of the Chlorophyll, Flavonoid and Protein Content of Microgreen Ricegrass (*Oryza sativa* L.) using Liquid Organic Fertilizer

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ABSTRACT

Microgreen ricegrass, recognized for its numerous health advantages, can be ingested as fresh juice due to its rich chlorophyll, flavonoids, and protein content. This study investigates whether applying Liquid Organic Fertilizer (LOF) enhances protein, flavonoids, and chlorophyll levels in microgreen ricegrass. The experimental design employed a non-factorial randomized approach, utilizing LOF concentrations of 0 ml/water, 5 ml/water, 10 ml/water, and 15 ml/water. The seeds selected for this research were from the Inpari 24 Gabusan brown rice variety. The parameters measured included plant height (cm), wet weight (grams), chlorophyll content (mg/L), flavonoid content (mgQE/g), and protein content (µL/g). The findings indicate that the F3 treatment (15 mL/liter of water) resulted in an increase in plant height by 16.29 cm, wet weight by 232.55 grams, chlorophyll a by 33.08 mL/g, chlorophyll b by 41.16 mL/g, total chlorophyll by 74.23 mL/g, flavonoids by 37.32 mgQE/g, and protein by 0.12 µL/g. In conclusion, applying Liquid Organic Fertilizer at a concentration of 15 ml/liter of water significantly enhanced the plant height, wet weight, chlorophyll levels, flavonoid content, and protein concentration in microgreen ricegrass.

Keywords: *Chlorophyll, Liquid Organic Fertilizer, Concentration, Protein, Rice Variety*

1. INTRODUCTION

Microgreen ricegrass offers extraordinary health benefits, including antioxidant, anticancer, and anti-inflammatory properties. With a high glycemic index and many bioactive compounds such as metabolites, microgreens have become increasingly popular as functional foods in recent years.

Microgreens are generally consumed as fresh juice, as garnishes on steak dishes, and in salads. The consumption of microgreens has sharply increased as they are utilized at the level of functional foods, both as beverages and staple components in functional diets. The morphology of microgreens consists of well-developed cotyledon leaves, undeveloped true leaves, and a central stem (Partap et al., 2023). They are a type of green vegetable harvested at 7 to 14 days after germination (Bhaswant et al., 2023; Li et al., 2021), and they contain a high concentration of beneficial minerals and phytochemicals that promote health. Research by Xiao Z et al. (2012) indicates that 25 varieties of microgreens, such as spinach, red cabbage, and cilantro, contain carotenoids, ascorbic acid, tocopherols, and phylloquinone. Microgreens' nutritional content is also higher than mature leaves, according to the USDA National Nutrient Database. Adawiyah et al. (2020) noted in their review that microgreens have the potential to act as antiviral agents due to the phytochemicals that can strengthen the immune system. Ricegrass microgreen (*Oryza sativa* L.) is a young rice plant believed to have health benefits for humans. Ricegrass juice contains selenium (Se), which is considered a functional food that may reduce the risk of oxidative stress and chronic inflammatory diseases (Chomchan et al., 2018). It has also been reported that ricegrass juice contains molecules with antioxidant activities, such as phenolic acids, flavonoids, and anthocyanins. Anthocyanin content is a phenolic compound that falls under the flavonoid group (Widyawati et al., 2014; Dhaka, 2023). Applying Liquid Organic Fertilizer (POC) on ricegrass microgreens can fulfill plant nutrient requirements, with the assimilation outcomes utilized to enhance plant growth and development (Niss and Nik, 2017; Solihin et al., 2019). POC is a solution that plants can easily absorb through both roots and stomata due to its composition of organic waste and animal waste containing multiple elements (Nur et al., 2016; Fernándezet al., 2022). POC consists of macro and micronutrients, particularly N, P, K, and Mg. The presence of flavonoid compounds in plants is influenced by nitrogen availability (Deng et al., 2019; Jiet al., 2017). Potassium and phosphorus have also been demonstrated to enhance phenolic and flavonoid content in plants (Ahmad et al., 2018; Martínezet al., 2016). The Indonesian population highly seeks after green mustard microgreens due to their higher nutrient content compared to mature plants. Microgreens contain vegetable oils rich in protein, which are not typically found in mature vegetables. This is because vegetable plants are abundant in these compounds during their early stages, making the N nutrient element crucial for the vegetative phase of plants as it promotes chlorophyll biosynthesis in the leaves (Purbajanti et al., 2019; Gholizadeh et al., 2017). Amino acid and protein synthesis are accelerated when plants receive POC (Supartha et al., 2012; Satrio et al., 2023). Cultivating young rice plants using microgreens is closely linked to the role of the planting medium. The type and characteristics of the planting medium utilized will impact plant growth, chlorophyll levels, carotenoids, and flavonoids as the planting medium serves as a support system for plant growth, providing nutrients and water, and facilitating root development (Permatasari, 2021).

Based on this, the author conducted research utilizing liquid organic fertilizer, which significantly impacts the increase of chlorophyll, flavonoid, and protein content in microgreens ricegrass of the red rice variety Inpari 24 Gabusan.

2. MATERIAL AND METHODS

2.1 Research Site and Time

The research was conducted in the Medan Denai District of Medan City, at an elevation of 16 meters above sea level, located at the coordinates N: 98022'44.45" E: 020,3426,43", as shown in Figure 1.

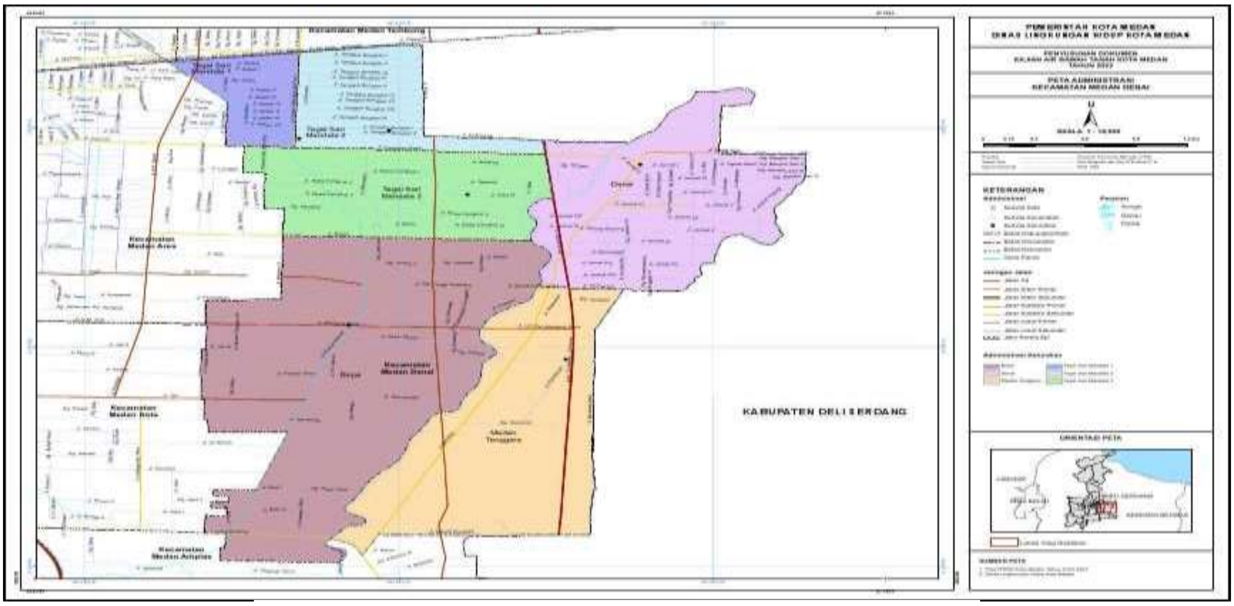

Figure 1. Administrative Map of Research Location

The materials used include red rice seeds of the Inpari 24 Gabusan variety, topsoil as the planting medium, water for irrigating the plants, POC as treatment, liquid nitrogen, 96% ethanol as a solvent, analytical-grade methanol as a solvent, sodium acetate as a reagent, aluminum chloride as a reagent, quercetin as a standard solution, aluminum foil, filter paper for extracting the solution, Coomassie Brilliant Blue (CBB) as a dye for measuring protein content, extra buffer solution, distilled water, bovine serum albumin (BSA) for the standard solution, polyvinyl pyrrolidone (PVP), and other supporting materials. The POC contains N 4.15%; P_2O_5 4.45%; K_2O 5.66%; organic carbon 9.69%; Mg 2.82%; Mn 193.1%; Cu 1179.8%; Zn 1986.1%; B 806.6%; Fe 505.5 ppm; Co 8.4 ppm; Mo 2.3 ppm; Pb 4.7 ppm; Cd 0.1 ppm; Hg <0.01 ppm; As 0.03 ppm; E. Coli <3.0 MPN/ml, pH 6.68, gibberellin, cytokinin, auxin.

The equipment utilized in this study includes seedling containers for planting, sprayers for irrigation, stationery, analytical scales for sample measurement, blenders for sample grinding, 20 cc urine pots for sample collection, beaker glasses for holding solutions, measuring cups, test tubes, 10 ml and 5 ml volumetric flasks, droppers and micropipettes for solution transfer, vortex mixers for solution homogenization, centrifuges for the separation of solutions and samples, steam cups, watch glasses for weighing samples, water baths for extract evaporation, spatulas for sample and material collection, and UV/Vis (Ultra Violet/Visible) spectrophotometers for assessing sample absorbance at specified wavelengths, along with liquid nitrogen tubes and various other supporting instruments.

This research used a non-factorial Randomized Block Design (RAK) comprising 5 replications and 20 experimental units, with each seedling container containing 1064 seeds. The POC concentrations applied were P0 = 0 ml/l water, P1 = 5 ml/l water, P2 = 10 ml/l water, and P3 = 15 ml/l water.

2.2 Research Implementation

Prepare 20 seedling containers filled with topsoil. Then, prepare 1,064 seeds and soak them in water for 10-12 hours per seedling container. The plants should be maintained through weeding and daily watering according to conditions. Liquid organic fertilizer (POC) is applied with one spraying four days after planting, following the treatment protocol, and harvesting is conducted 11 days after planting. Following planting, the height of the plants is measured nine days after sowing.

Making Liquid Organic Fertilizer: The necessary tools for creating POC have been readied, including a 120-liter plastic bucket for the POC container, a knife and scissors for cutting the ingredients, and a 1-liter Philips blender for blending the ingredients. The required ingredients have also been prepared, such as 8 kg of Moringa oleifera leaves, 2.5 kg of banana leaves, 4 kg of banana stems (*Musa paradisiaca* L.), 1 kg of cassava leaves (*Manihot esculenta*), 2 liters of Effective microorganism brand EM4, 2.5 liters of molasses, 6 liters of rice washing water, 5 liters of coconut water, and 100 liters of water. Blend the Moringa, cassava, banana, and banana stems, then place them in a container. Add rice washing water, EM4, and molasses, and stir in water until well mixed. Seal the container tightly and store it in a cool (dark) place for fermentation. Wait for approximately 1 month (28 days) until the solution turns brownish, shows no signs of insect infestation, and does not have a strong odor but rather a faint tape-like aroma, indicating the successful creation of liquid organic fertilizer.

Chlorophyll Analysis: Chlorophyll content analysis was conducted using the Wintermans and De Monts method (1965) 11 days after planting (HST). Chlorophyll was extracted by grinding the leaves with 96% ethanol. The mixture was filtered through filter paper until a leaf extract volume of 25 ml was obtained. Measurements were taken using a UV/Vis spectrophotometer at wavelengths of 649 nm (chlorophyll b) and 665 nm (chlorophyll a), with 96% ethanol serving as a blank for neutralization. The chlorophyll content was calculated using the following formulas: Chlorophyll a = (13.36 x A665) – (5.19 x A649); Chlorophyll b = (27.43 x A649) – (8.12 x A665); Total chlorophyll = (5.24 x A665) + (22.24 x A649), with units expressed in mg/L.

Flavonoid Analysis: The Colorimetric method with AlCl3 reagent was used to analyze the total flavonoid content, with aluminum chloride and quercetin as a comparator (Quercetine Equivalent/QE). A stable complex of flavone compounds was formed by aluminum chloride, causing absorption of electromagnetic radiation. A standard quercetin solution was prepared by dissolving 10 mg of quercetin in 100 ml of methanol. A 2 ml of 100 pmm quercetin solution was pipetted and 0.1 ml of $AlCl_3$, 0.1 ml of CH_3COONa, and 3 ml of distilled water were added. The solution was then homogenized and incubated for 25 minutes. The absorbance of the solution was measured with a UV/Vis spectrophotometer at a wavelength of 427 nm every 1 minute until stable. Subsequently, different volumes of quercetin solution were taken and put into a 5 ml measuring flask, and the solution concentration was adjusted to 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. Each solution was then incubated for 25 minutes after adding $AlCl_3$, CH_3COONa, and distilled water, and the absorbance was measured on a UV/Vis spectrophotometer at a wavelength of 427 nm. A quercetin calibration curve and the linear regression line equation y = ax + b were obtained. The total flavonoid levels were determined by weighing 10 mg of sample extract, and dissolving it in 10 ml of methanol to obtain a concentration of 1000 ppm. Then, 2 ml of the solution was taken, and 0.1 ml $AlCl_3$, 0.1 ml CH_3COONa, and 3 ml of distilled water were added. The solution was homogenized and incubated for 25 minutes, and the absorbance was measured on a UV/Vis spectrophotometer at a wavelength of 427 nm.

Soluble Protein Analysis: The Bradford method (1976) was utilized to analyze the soluble protein. The Bradford solution was prepared by dissolving 0.02 g of Comassie Brilliant Blue (CBB) in 10 ml of 95% ethanol and 20 ml of phosphoric acid. The solution was then filtered in the dark using filter paper, dissolved in 150 ml of distilled water, and diluted five times before absorbance measurements were conducted. To create a stock solution with a concentration of 1000 ppm, 0.01 g of Bovine Serum Albumin (BSA) was weighed and dissolved in 10 ml of distilled water. Dilution was achieved using the stock solution to create solutions with varying lower concentrations ranging from 0-100 ppm. Using this method, the measurement of dissolved protein involved weighing a 0.1 g sample, adding 1% PVP, grinding it with liquid N2, and introducing 1 ml of extract buffer into the tube. The mixture was then centrifuged at 1000 ppm at 4oC for 10 minutes. Subsequently, 50µL of the sample was placed into a test tube, 2.5 ml of Bradford solution was added, and the solution was incubated for 10-60 minutes in the dark. The absorbance was measured using a UV/Vis spectrophotometer at 595 nm. This UV-Vis spectrophotometry method is an analytical technique that utilizes UV light at a wavelength of 100-400 nm.

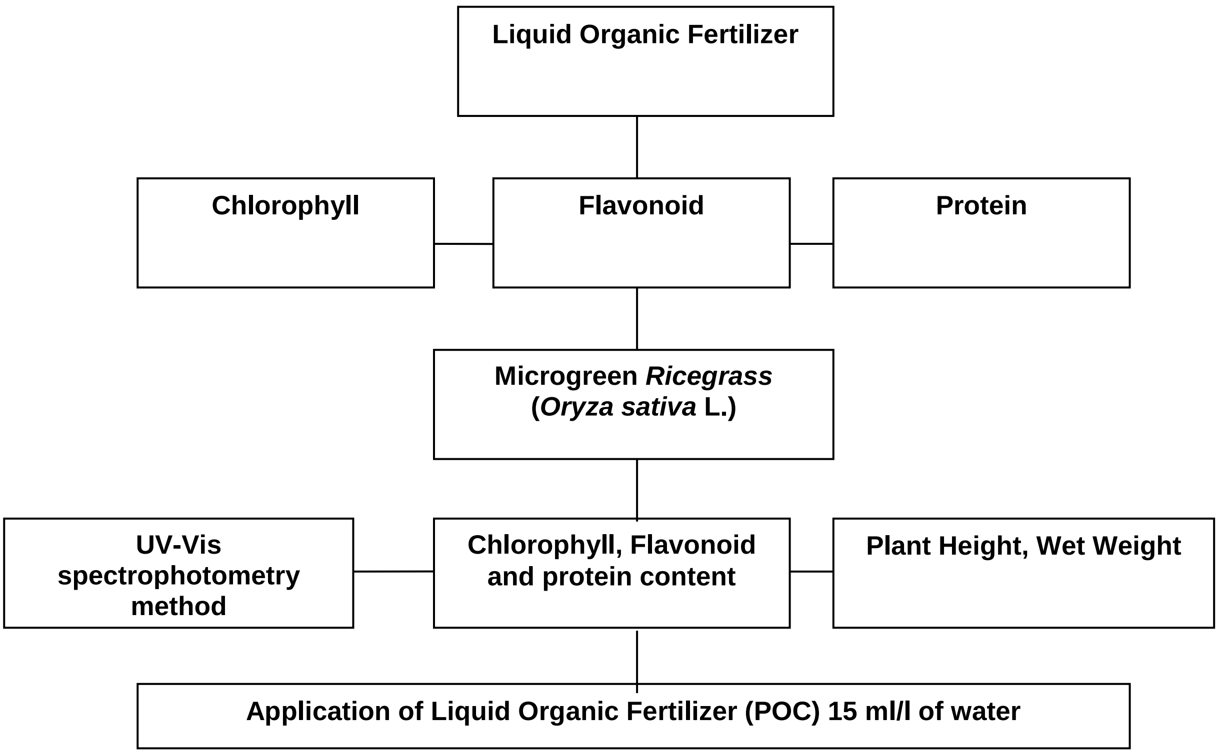

Figure 2. Flowchart of Ricegrass (*Oryza sativa* L.) Microgreen Research

3. RESULT AND DISCUSSION

The results of the Microgreen Ricegrass (*Oryza sativa* L.) research using Liquid Organic Fertilizer 11 days after planting can be seen in Figure 1.

3.1 Plant Height

The results of the research on the height of Microgreen Ricegrass (*Oryza sativa* L.) plants using Liquid Organic Fertilizer can be seen in Table 1.

Table 1 demonstrates that the treatment of 15 ml/liter of water and the treatment of 0 ml/liter of water yielded significantly different outcomes in the height of the ricegrass plant at 16.29 cm. This discrepancy is attributed to the liquid nature of POC, which facilitates the absorption of nutrients by plants and fulfills their nutritional needs. This is consistent with the findings of Niss and Nik (2017), which indicate that POC is more readily absorbed by plants through roots and stomata, satisfying the plant's nutrient requirements and promoting growth and development. Similarly, Putra et al. (2020) assert that liquid organic fertilizer contains both macro and micronutrients and beneficial microorganisms that enhance the process of water absorption in optimal rice seeds, resulting in rapid plant growth. The research of Muthu et al. (2023) and Hooks et al. (2022) further supports these conclusions by demonstrating that liquid organic fertilizer is just as effective as commercial liquid fertilizer in providing plants with the necessary nutrients for growth, making it an essential source of nutrition for plant development.

Figure 3. Ricegrass 11 days after planting

Table 1. Average Height of Ricegrass Plants

POC Concentration	Average (cm)±SD
(0 ml/l water)	15,18bc±0,63
(5 ml/l water)	15,41abc±0,30
(10 ml/l water)	15,43ab±0,40
(15 ml/l water)	16,29a±0,31

Notes: Different numbers and letters in the columns indicate significant differences according to the Duncan Test at the 5% test level.

3.2 Plant Wet Weight

The results of the Microgreen Ricegrass (*Oryza sativa* L.) research using Liquid Organic Fertilizer on the wet weight parameters of plants can be seen in Table 2.

Table 2. Average Wet Weight of Ricegrass

POC Concentration	Average (cm)±SD
(0 ml/l water)	190,11c±20,40
(5 ml/l water)	216,85b±8,39
(10 ml/l water)	220,91b±5,21
(15 ml/l water)	232,55a±6,51

Notes: Different numbers and letters in the columns indicate significant differences according to the Duncan Test at the 5% test level.

As indicated in Table 2, treatment P3 (15 ml/l water) yielded the most favorable outcome, with a ricegrass wet weight of 232.55 grams. The presence of POC provides essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K) that are crucial for plant growth. This finding aligns with the research conducted by Jiaying et al. (2022), which emphasizes that adequate levels of N, P, and K can enhance the growth and development of rice seedlings by improving photosynthetic efficiency and energy production, thereby elevating the overall energy status. Applying water-soluble POC facilitates nutrient absorption and enhances photosynthesis during the vegetative stage of ricegrass. Furthermore, Levinsh (2023) highlighted water's significant role in transportation and transpiration within plants. Mustofa et al. (2022) reported that using POC resulted in the highest fresh weight per plant in pak choi. This is consistent with the findings of Hooks et al. (2022), which demonstrated that vegetable plants treated with liquid organic fertilizers containing microbial agents achieved superior fresh and dry shoot weights compared to control groups.

3.3 Chlorophyll

The results of the Microgreen Ricegrass (*Oryza sativa* L.) research using Liquid Organic Fertilizer on the parameters of chlorophyll a, b, and total content can be seen in Tables 3., 4., 5.

Table 3. Average Chlorophyll a Ricegrass

POC Concentration	Average (cm)±SD
(0 ml/l water)	30,99c±1,16
(5 ml/l water)	31,86bc±0,71
(10 ml/l water)	31,46bc±0,52
(15 ml/l water)	33,08a±0,19

Notes: Different numbers and letters in the columns indicate significant differences according to the Duncan Test at the 5% test level.

Table 4. Average Chlorophyll b of Ricegrass

POC Concentration	Average (cm)±SD
(0 ml/l water)	28,5bc±5,35
(5 ml/l water)	32,06bc±5,84
(10 ml/l water)	34,78abc±3,98
(15 ml/l water)	41,16a±2,38

Notes: Different numbers and letters in the columns indicate significant differences according to the Duncan Test at the 5% test level.

Table 5. Average Total Chlorophyll of Ricegrass

POC Concentration	Average (cm)±SD
(0 ml/l water)	59,5c±5,65
(5 ml/l water)	63,92bc±5,21
(10 ml/l water)	66,24b±4,01
(15 ml/l water)	74,23a±2,43

Notes: Different numbers and letters in the columns indicate significant differences according to the Duncan Test at the 5% test level.

According to the data presented in Tables 3, 4, and 5, the water treatment with 15 ml/l produced notable levels of chlorophyll, with chlorophyll a at 33.08 mL/g, chlorophyll b at 41.16 mg/L, and total chlorophyll at 74.23 mg/L. The levels of chlorophyll in ricegrass are heavily influenced by the nutrients found in POC, particularly N, P, and K, as indicated by Agustien and Suhardjono's literature review (2016). The findings align with Hendriyani's literature (2018), which suggests that chlorophyll content is typically higher during the vegetative phase, such as when ricegrass microgreens are harvested 11 days after planting. The age of the plant leaves plays a significant role in determining the chlorophyll level, as Kalaji et al. (2018) stated. Furthermore, Hooks et al. (2022) found that the relative chlorophyll and anthocyanin content in leaf tissue increased in plants that were given liquid organic fertilizer.

3.4 Flavonoid Compounds

The results of the Microgreen Ricegrass (*Oryza sativa* L.) research using Liquid Organic Fertilizer on flavonoid compound parameters can be seen in Table 6.

The treatment with a concentration of 15 ml/l water exhibited the highest flavonoid content at 37.32 mgQE/g, as detailed in Table 6. It is hypothesized that POC contains nitrogen nutrients that play a role in influencing flavonoid compounds. According to Zhao et al. (2021), nitrogen levels can impact plant secondary metabolism, including the production of flavonoid compounds. This finding aligns with the research by Deng et al. (2019), which suggests that nitrogen availability affects the presence of flavonoid compounds in plants. Additionally, Amarowicz et al. (2020) have highlighted that optimal nitrogen fertilization and selecting appropriate plant varieties can influence the formation of beneficial biologically active compounds, such as phenolics. These findings are further supported by Xiao et al. (2019), who reported that Brassica Microgreens are rich in phytochemicals, particularly total phenolics. Zhao et al. (2021) emphasized the importance of nitrogen as a crucial nutrient for plants, with a significant impact on flavonoid content.

Table 6. Average Flavonoid Compounds of Ricegrass

POC Concentration	Average (cm)±SD
(0 ml/l water)	26,65c±3,04
(5 ml/l water)	31,16b±2,27
(10 ml/l water)	32,43b±1,68
(15 ml/l water)	37,32a±0,72

Notes: Different numbers and letters in the columns indicate significant differences according to the Duncan Test at the 5% test level.

3.5 Protein

The results of the Microgreen Ricegrass (*Oryza sativa* L.) research using Liquid Organic Fertilizer on protein content parameters can be seen in Table 7.

Table 7. Average Dissolved Protein of Ricegrass

POC Concentration	Average (cm)±SD
(0 ml/l water)	0,07bc±0,03
(5 ml/l water)	0,08bc±0,03
(10 ml/l water)	0,09ab±0,01
(15 ml/l water)	0,012a±0,01

Notes: Different numbers and letters in the columns indicate significant differences according to the Duncan Test at the 5% test level.

The data presented in Table 7 indicates that the P3 treatment, which consisted of 15 ml/l water, yielded the highest protein content at 0.12 µL/g. POC can provide essential nutrients, such as N, P, and K, needed by plants to increase protein content in ricegrass microgreens. High nitrogen levels in the soil can increase protein levels in plants, a concept supported by Zhao et al. (2021), who stated that nitrogen plays a role in forming amino acids, which are essential for protein synthesis. Potassium (K) is also crucial for biochemical processes related to protein synthesis, carbohydrate metabolism, and enzyme activity, as Hasanuzzaman et al. (2018) noted. Mlinarić et al. (2023) also reported that plant proteins tend to have fewer essential amino acids than animal proteins, with methionine, lysine, and tryptophan being the primary exceptions. These findings align with the report of Kyriacou et al. (2020) in Alloggia et al. (2023), which states that organic waste-based substrates have been shown to increase chlorophyll, carotenoid, and total phenolic content in pakchoi (Brassica rapa L. subsp. chinensis).

4. CONCLUSION

Applying Liquid Organic Fertilizer (LOF) at a concentration of 15 ml/l of water significantly increases the height of plants, wet weight, chlorophyll content, flavonoids, and protein levels in microgreen ricegrass.

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